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Time Resolved X-ray Absorption Spectroscopy Studies of Bacterial Alcohol Dehydrogenase Reveal Mechanistic Information Beyond Kinetics

O.Kleifeld (Weizmann Institute of Science), A. Frenkel (Yeshiva U.), and I. Sagi (Weizmann Institute of Science)

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We have developed time-dependent X-ray absorption spectroscopy (XAS) procedures to directly follow the structural dynamic changes in metal ion centers during metalloenzyme catalysis. The zinc dependent alcohol dehydrogenase metalloenzymes catalyze the reversible oxidation of a wide range of aliphatic and aromatic alcohols to their corresponding aldehydes and ketones using NAD⁺ or NADP⁺ as a coenzyme. The reaction mechanism of alcohol dehydrogenase catalysis has been the focus of intensive mechanistic studies involving structural and spectroscopic approaches, yet the actual mechanism is still under debate. Specifically, there is no clear agreement about the coordination environment of the metal ion and the reaction intermediates during turnover. Using a combined stopped-flow and freeze-quench technology together with XAS we have trapped various transient intermediate states of the ternary enzyme-coenzyme-substrate complexes in millisecond time scales. Time resolved XAS data analysis reveals significant structural changes at the metal center during enzyme turnover that are correlated with the pre-steady state kinetic profile of the enzyme. Interestingly, these changes include a change in coordination number upon substrate binding and unexpected concomitant changes in the first shell metal-ligand bond distances with time. We propose a refined reaction mechanism model for alcohol dehydrogenase catalysis that outlines the intimate communication of the catalytic zinc ion with its neighboring ligands during catalysis.